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MERCURY IN AQUATIC SYSTEMS: METHYLATION, OXIDATION-REDUCTION, AND BIOACCUMULATION

Harvey W. Holm, et al

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August 1974

MERCURY IN AQUATIC SYSTEMS:

METHYLATION, OXIDATION-REDUCTION, AND BIOACCUMULATION

by

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ROAP 21 AIM, Task 11
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ABSTRACT

The role of organisms in the fate of mercury in aquatic environments was evaluated. Objectives were (1) to quantitate transformations of mercury in water-sediment systems, (2) to investigate the fate of elemental mercury in microbial growth systems, and (3) to measure the concentration of total and methylmercury in food chain organisms.

In anaerobic water-sediment systems spiked with calcium acetate and mercuric chloride, elemental mercury was produced in larger quantities than methylmercury. The rate of methylation of mercury in aerobic environments was comparable to that in anaerobic environments; however, the rate of release of elemental mercury to the atmosphere during aerobic incubation was nearly three times that observed during anaerobic incubation. No dimethylmercury was produced in these systems.

In water-sediment systems, added elemental mercury was oxidized and deposited in the sediments where small amounts of methylmercury were formed. Six pure cultures of bacteria oxidized elemental mercury, but none formed methylmercury. Two Pseudomonas species did not grow in the presence of elemental mercury.

In a stream receiving mercuric ion, mosquito fish contained more methylmercury than did tadpoles, snails, and aquatic insects. Algae did not contain methylmercury, even though their total mercury levels were high.

This report was prepared in fulfillment of ROAP 21AIM, Task 11, by the Freshwater Ecosystems Branch, Southeast Environmental Research Laboratory, National Environmental Research Center-Corvallis, U. S. Environmental Protection Agency. Work was completed as of June 30, 1974.

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SECTION I

CONCLUSIONS

In water-sediment systems receiving mercuric ion or elemental mercury, the methylmercury content was always less than 1.0% of the total mercury; in pure culture studies no methylmercury was formed.

Elemental mercury was produced in much larger quantities than methylmercury in water-sediment systems receiving mercuric ion. The rate of release of elemental mercury to the atmosphere during aerobic incubation was nearly three times the rate observed during anaerobic incubation.

Dimethylmercury was not produced in anaerobic or aerobic water-sediment systems.

Elemental mercury was oxidized in pure cultures of Escherichia coli, Pseudomonas fluorescens, Pseudomonas aeruginosa, Citrobacter, Bacillus megaterium, and Bacillus subtilis, and the oxidized mercury accumulated on the bacterial cells.

Of the six cultures tested, only the two Pseudomonas cultures were inhibited by elemental mercury.

Mosquito fish contained significantly more methylmercury than did tadpoles, snails, and aquatic insects collected from the same water. Algal masses did not contain methylmercury.

SECTION II

RECOMMENDATIONS

Elemental mercury, shown here to be a significant intermediate product of water-sediment systems containing mercury, should be intensively studied to determine its role in the fate of mercury in aquatic systems. Factors to consider are (1) the transport of elemental mercury to important food chain organisms, such as fish, and its possible methylation within the animal; (2) the rate of loss of elemental mercury from polluted systems so that accurate predictions can be made concerning the recovery of polluted waters, and (3) the transport of elemental mercury from the atmosphere to terrestrial and aquatic environments.

Methylmercury accumulates in fish although it cannot be detected in natural waters with the analytical methodology now available. Therefore techniques to measure environmental levels of methylmercury must be developed to allow insight into the mechanisms of mercury transport.

The phenomenon of methylation of mercury should be explored in detail. Because this work showed that bacteria form only small amounts of methylmercury, if any, other organisms such as fungi, aquatic insects, and fish should be evaluated for their abilities to methylate various forms of mercury.

SECTION III

INTRODUCTION

Mercury in the environment has been the focus of intensive research, primarily because of the well-known presence of methylmercury in fish from mercury-contaminated waters[1, 2, 3, 4, 5]. Since the methylated form of mercury has been shown to be toxic to animal life in small amounts, its formation, degradation, and concentration in organisms are of great interest.

Methylmercury can be formed in complex laboratory growth systems[6, 7, 8]. Therefore, bacteria are thought to be the organisms responsible for the methylation of mercury in the environment. Methylmercury is assumed to be formed in the sediments, released to the water, and accumulated by the fish either through direct uptake or a food chain or both[3, 9, 10]. However no data are available to clearly support either mechanism.

Evidence is needed to define the role of bacteria in the methylating phenomenon. To date only two laboratories[11, 12] have found pure bacterial cultures that methylate mercury, and these only in small amounts. However, cell-free extracts of methane bacteria have been reported to methylate mercury[13]. To complicate matters, Spangler[14] reported that methylmercury is degradable by bacteria, suggesting that it may not accumulate in the environment.

The mechanism of transport of methylmercury to fish is also difficult to determine, in part because the compound has not been detected in natural waters or sediments in concentrations great enough to cause problems to the fish[15]. In his study using a stream into which mercury had been discharged, Uthe[10] showed that rainbow trout held in cages in the stream, after discharge was stopped, accumulated methylmercury in their bodies. These researchers abandoned total mercury measurements for water samples because the levels of mercury were below the detection limits of the methodology used.

As an alternative to direct uptake of methylmercury, food chain transport to fish has been proposed[9, 16]. Again, few data are available to support this hypothesis in natural waters. Usually the data on food chain transport of methylmercury to fish have been obtained from short term experiments in which methylmercury is added to the systems and uptake rates are studied[17].

An alternative to the production of methylmercury in the environment by bacteria, and subsequent uptake by fish, is the uptake of inorganic mercury followed by methylation within the fish. Jernelov[18] suggests that organisms in fish slime may methylate mercury, but no data are presented in the report. Others[19] have demonstrated that fish liver homogenates can methylate mercury. To date, however, no one has reported that live fish can methylate inorganic mercury.

If inorganic mercury is to be taken up and methylated by fish, in many aquatic environments it must first be released from the sediment sinks into the water column. Elemental mercury, a product of microbial action on mercurials, could be the transport form. Pure cultures of bacteria can produce elemental mercury from mercuric chloride[20], phenylmercuric acetate[21], ethylmercuric phosphate[21], methylmercuric chloride[21], and methylmercuric bromide[22]. Over 70% of mercury added as mercuric ion can be released by mixed bacterial cultures[7] as elemental mercury; smaller amounts can be released from streams[23] and sediments[24].

Information is lacking on several points:

- the relative importance of each mercury transformation product (methylmercury, dimethylmercury, and elemental mercury);
- the microbial fate of elemental mercury; and
- the contribution of methylmercury in food chain organisms to fish.

This study was designed to define environments that produce mercury transformations; to identify mercury products and the rates of their formation; and to complete a food web study to determine total mercury and methylmercury levels and distribution in organisms other than fish.

SECTION IV

MATERIALS AND METHODS

MATERIALS AND ANALYTICAL PROCEDURES

Organisms

Mixed bacterial populations present in local pond sediments were used as inocula for studying the fate of mercury in water-sediment systems. No attempt was made to isolate and identify the bacterial flora present in the sediments.

Pure cultures of *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Citrobacter*, *Bacillus megaterium*, and *Bacillus subtilis* were used for studying the transformations of elemental mercury. Inocula of the *Bacillus* species were 24-hour cultures (25°C, 125 rpm) grown in a basal salts medium[25] containing 0.1% yeast extract; the other four cultures were grown on the basal salts medium containing 0.25% glucose.

Ten different families of aquatic organisms were collected by personnel at the Savannah River Ecology Laboratory, Aiken, South Carolina, from a small stream receiving low levels of mercuric chloride. Organisms from a control stream and from the test stream two weeks after the mercury input was discontinued were also collected. Water was drained from the organisms and the organisms were homogenized in a chilled tissue grinder prior to analysis.

Reagents

Reagent grade chemicals were used for the preparation of the growth media. Extractions of methylmercury were completed with spectroanalyzed grade solvents.

Growth Measurements

During the course of experiments, bacteria were counted by plating serial dilutions, in duplicate, in Tryptone Glucose Extract Agar (TGE) pour plates, incubating at 25°C for 48 hours, and counting the colonies.

Analytical Procedures

A Laboratory Data Control (LDC) UV Monitor, Model 1235 was used for quantitating total mercury, mercuric ion, and elemental mercury.

For total mercury, each sample was digested with aqua regia and oxidized with permanganate[26]. Aliquots of the digest were pipetted into a flask and reacted with a reducing agent, stannous chloride, for 30 seconds. The mercury, all in the form of elemental mercury, was determined using a cold vapor technique[27].

Elemental mercury was quantitated by using the cold vapor technique on the sample directly (without digestion and without the reducing agent).

An aliquot of undigested sample was reacted for 30 seconds with the reducing agent and analyzed with the cold vapor technique, yielding a value for the combined concentrations of mercury present as elemental mercury and mercuric ion. The difference between the amount of mercury recovered with and without the reducing agent was considered to be mercuric ion.

Reference curves from digested and undigested standards having an average coefficient of variation of 5% were used to quantitate duplicate mercury analyses.

In this laboratory, for sediments spiked with mercuric ion, recovery was 95%.

Methylmercury was extracted from fish, sediments, media, and aquatic biota by the method of Longbottom et al.[28] and quantitated with a Barber-Coleman gas chromatograph equipped with a Radium-226 electron capture detector. A 1 m x 6 mm Pyrex column packed with 5% DEGS on 80-100 mesh Chromosorb W coated with 5% KBr was employed for separation. The nitrogen carrier gas flow was 60 ml/min.; the operating temperatures for the column, detector, and inlet were 140°, 210°, and 180°C, respectively.

Recoveries of methylmercury hydroxide added to sediments averaged 67%. A linear calibration curve was obtained using methylmercuric bromide (0.01 to 1 ng mercury).

Release of elemental mercury from growth systems was determined by bubbling effluent gases through two traps (Figure 1)[29]. The first trap, containing phosphate-carbonate, removes volatile organomercury compounds such as ethyl and methylmercuric chloride; the second trap, containing acid permanganate, removes elemental mercury from the effluent gas.

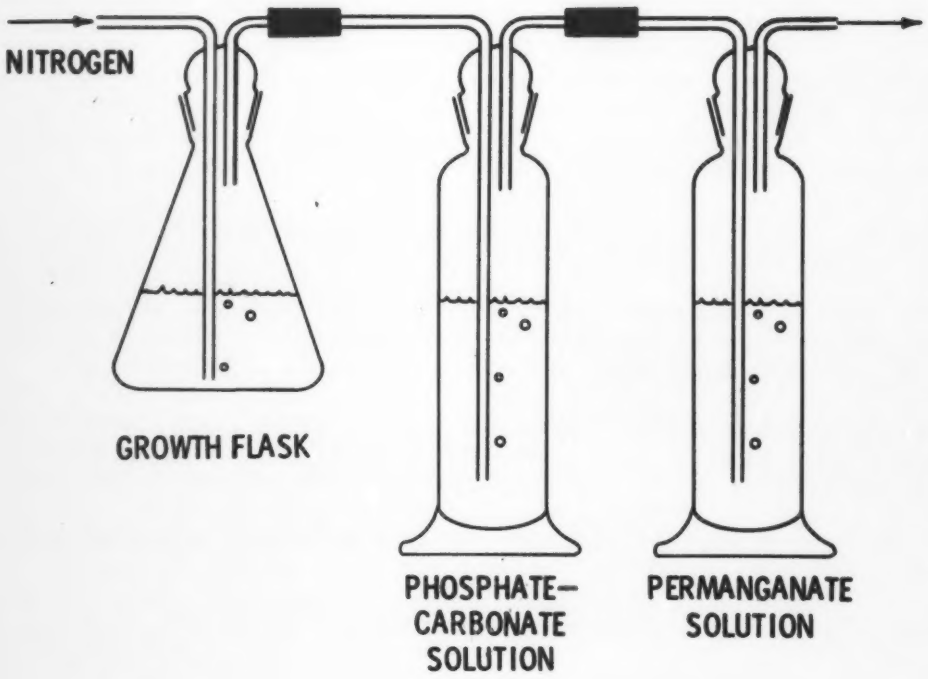

Figure 1. Growth system with mercury traps[29]

phosphate-carbonate trap replaced by an acidified mercuric chloride trap. Since dimethylmercury is cleaved to form monomethylmercury in the presence of excess mercuric ion, this trap should quantitatively trap dimethylmercury as monomethylmercury. The methylmercury in the trap was quantitated by Longbottom's method[28].

A Varian Aerograph Model 90P-3 chromatograph, equipped with a thermal conductivity detector operated at room temperature was used to measure production of methane and carbon dioxide. Separation was obtained using a 1.2 m x 6 mm glass column packed with Porapak Q.

EXPERIMENTAL DESIGN

Each phase of this research required a distinctly different approach. Only an outline of the experimental design will be documented here; comprehensive descriptions of the experiments may be found in Appendices A, B, and C.

Phase I -- Transformations of Mercury in Water-Sediment Systems (See Appendix A)

The fate of mercury (mercuric ion and elemental mercury) was determined in laboratory water-sediment systems bubbled with either nitrogen gas or air (flow rate, 20 ml/min).

Mercury forms quantitated in the effluent air, water, or sediments, as appropriate, included total mercury, mercuric ion, elemental mercury, methylmercury, and dimethylmercury.

Transformations of mercury in the systems were monitored as functions of

(1) calcium acetate concentration;

(2) mercuric ion concentration;

(3) form of mercury added to the systems; and

(4) incubation conditions (anaerobic or aerobic).

Phase II -- Transformations of Elemental Mercury in Microbial Growth Systems (See Appendix B)

Six pure cultures of bacteria were used to study the fate of elemental mercury in microbial growth systems.

Elemental mercury was equilibrated[30] over a 48-hour period with a sterile basal salts medium[25] containing either 0.25% glucose or 0.1% yeast extract.

At zero time (just prior to inoculation), duplicate liquid samples were removed for mercury analyses (total mercury, mercuric ion, elemental mercury), after which bacteria were added, the flask contents were mixed, and samples were removed for plate counts.

After a 48-hour incubation (25°C, 125 rpm) mercury analyses (elemental mercury, mercuric ion, total mercury, methylmercury, and cell-associated mercury) and bacterial counts (TGE agar pour plates) were completed.

Phase III -- Mercury Distribution in Aquatic Biota (See Appendix C)

The objective of this study was to determine the concentration of methyl- and total mercury in a variety of aquatic organisms exposed to a low level of mercuric ion over an extended time period. The mercury levels of these biota were compared to mercury levels in similar biota taken from a control area receiving no known mercury input. Organisms that were analyzed included dragonfly and damselfly nymphs, beetles, water bugs, snails, tadpoles, and mosquito fish, Gambusia affinis. Analyses were performed on homogenized whole organisms.

SECTION V

RESULTS AND DISCUSSION

PHASE I -- TRANSFORMATIONS OF MERCURY IN WATER-SEDIMENT SYSTEMS

Transformations of mercury in various systems (Appendix A) were monitored as functions of (1) acetate content, (2) mercuric ion concentration, (3) incubation conditions (aerobic or anaerobic), and (4) the form of mercury added to the system.

Methane bacteria produce large amounts of methylcobalamine, a compound that has been implicated[13, 31, 32] in the methylation of mercury. These strictly anaerobic organisms are probably therefore involved in this reaction in sediments. Water-sediment systems, containing calcium acetate (Appendix A) to optimize the growth of these anaerobes, were used to study the transformations of mercury.

In 25-day anaerobically incubated systems, production of methylmercury was stimulated by high concentrations of mercuric chloride and calcium acetate (Table 1). In systems receiving only mercuric chloride, the concentration of methylmercury was no higher than that of the control. This suggests that the methane bacteria, which require short chain fatty acids such as acetate for growth, could be involved in the methylation of mercury in sediments. However, investigators have been unable to demonstrate that pure cultures of methane bacteria methylate mercury[11].

Autoclaved systems containing high acetate and mercuric chloride concentrations did not form methylmercury, again suggesting that viable bacteria are responsible for the production of methylmercury in sediments.

Elemental mercury was the predominate product formed in these 25-day anaerobically incubated systems (Table 1). More elemental mercury than methylmercury was produced. As with methylmercury, highest amounts of elemental mercury were released from systems containing high concentrations of acetate and mercuric chloride. Elemental mercury is probably formed both biotically[20] and abiotically[24] in these systems, although acetate definitely increased the output of elemental mercury.

The production of elemental mercury is expected since the Eh of anaerobic environments that is optimum for growth of methane bacteria is low and permits the reduction of

Table 1. ANAEROBIC MERCURY TRANSFORMATION IN WATER-SEDIMENT SYSTEMS
(25-Day Incubation)

System	Elemental Mercury (µg/g sediment)	Methylmercury (µg/g sediment)
Control	0.0	0.003
50 mg/l $HgCl_2$	0.012	0.002
50 mg/l $HgCl_2$ + 10 g/l acetate	0.540	0.028
10 mg/l $HgCl_2$ + 10 g/l acetate	0.052	0.009

mercuric ion. Methylmercury, when found in natural sediments, is always present in small amounts, usually <0.1% of the total mercury[22, 23].

The effects of changing from anaerobic to aerobic conditions on the fate of mercury in water-sediment systems were examined in a system incubated anaerobically for 14 days, and then aerobically for 14 days (Appendix A). The system contained mercuric chloride (50 mg/l) and calcium acetate (10 g/l), which together were shown to result in production of elemental mercury and methylmercury in anaerobic systems.

Aeration of the system initially changed the concentrations of the methylmercury that had been produced during the 14-day anaerobic incubation (Figure 2). The decrease in concentration may be a result of microbial demethylating action[22], since methylmercury is not readily degraded abiotically in water[34].

By the end of the aerobic incubation, the methylmercury concentration in the sediment had increased to 60 ng/gram of sediment. The average rate of methylmercury formation over both the 14-day anaerobic and the 14-day aerobic incubation periods was approximately 5 ng/g sediment/day. However methane, indicating the action of methane bacteria, was produced during the anaerobic incubation but was not detected during the aerobic incubation (Table 2). Probably two physiologically different bacterial populations (one anaerobic, the other aerobic) were involved in the methylation of mercury during the 28-day incubation.

Surprisingly, aeration stimulated the release of elemental mercury from the water-sediment system (Figure 3). About 3 mg (1.2%) of the added mercuric ion was released during the 28-day incubation at a rate of 60 ng/gram sediment/day during the anaerobic period, and at a rate of 160 ng/gram sediment/day during the aerobic period. Microbial activity, which greatly increased upon aeration, probably mediated the reduction of mercuric ion to elemental mercury. The rate of release of elemental mercury was not constant during the aerobic incubation; the rate decreased significantly from day 14 to day 28, probably reflecting a decreasing rate of microbial metabolism.

The concentration of soluble mercury, which was not methylmercury, increased in the water-sediment system during aeration (Table 3). Although mercuric chloride was added initially at a concentration of 50 mg/l, during anaerobic incubation it was detected in the sediment-free water at

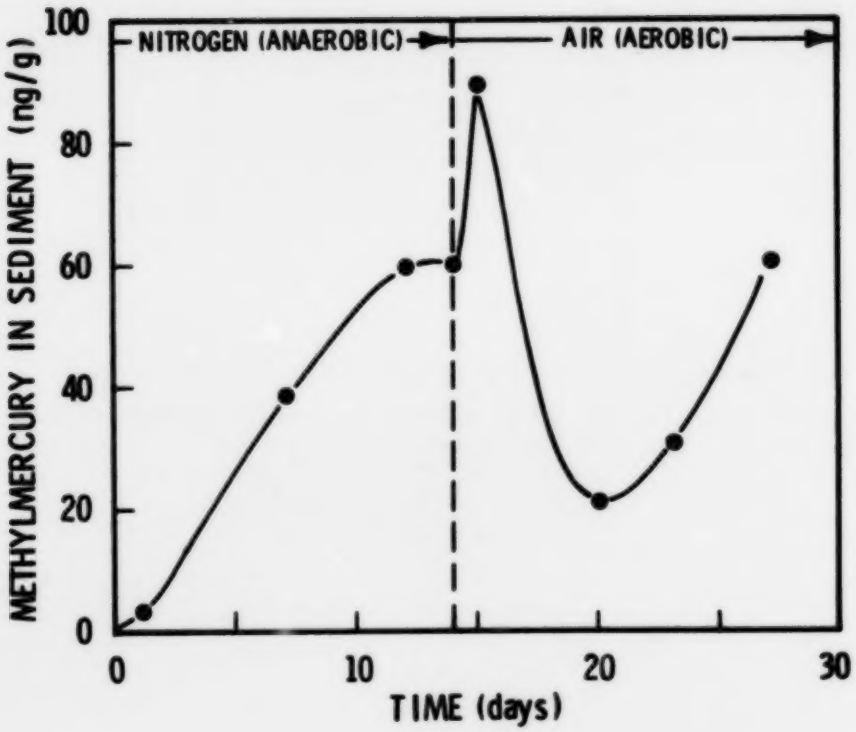

Figure 2. Production of methylmercury in water-sediment systems.

Table 2. RELATIVE CONCENTRATIONS[a] OF METHANE AND CARBON DIOXIDE IN ANAEROBIC AND AEROBIC INCUBATION PERIODS

Time	Methane	Carbon Dioxide
Anaerobic (Day 13)	35	15
Aerobic (Day 15)	0	244

[a]Relative concentrations were determined by measuring peak areas after gas chromatography analysis.

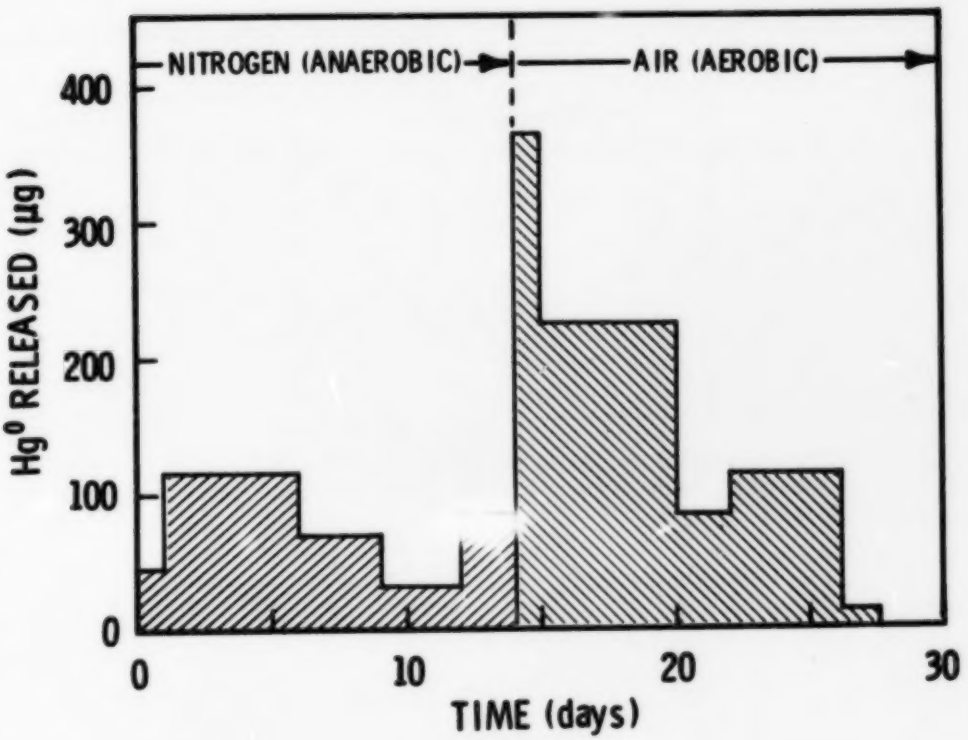

Figure 3. Production of elemental mercury in water-sediment systems. The value reported is the total amount of elemental mercury released from 1,000 grams of sediment each day.

Table 3. TOTAL DISSOLVED MERCURY IN ANAEROBIC AND AEROBIC INCUBATION PERIODS[a]

Days	Total Mercury, µg/l	
	Anaerobic	Aerobic
2	3	
13	15	
15		80
27		46

[a]Mercury analyses were completed on water samples in which the bacteria were removed by centrifugation (18,000 XG for 15 minutes).

only 3-15 µg/l. During aerobic incubation, however, it increased to 45-80 µg/l. The sediment obviously acted as a sink for mercury.

Our experiments show that elemental mercury is released from water-sediment systems receiving mercuric ion. However, when mercury in the elemental form is added to water-sediment systems from an atmospheric source (Appendix A), it oxidizes in the system and accumulates in sediments (Table 4). A 4-day exposure of the system to elemental mercury resulted in a 10-fold increase of mercury in the sediments; a 33-day exposure resulted in a mercury concentration in the sediments of 100 times that of the control.

The atmospheric additions of elemental mercury resulted in production of small amounts of methylmercury in the sediments (Table 4). However the amount of methylmercury produced was not greater than the amount obtained from the additions of mercuric chloride to sediments discussed in previous experiments.

PHASE II -- TRANSFORMATIONS OF ELEMENTAL MERCURY IN MICROBIAL GROWTH SYSTEMS

The objective of Phase II was to determine the fate and impact of elemental mercury in systems (Appendix B) containing pure cultures of bacteria. Phenomena examined included (1) the oxidation and methylation of elemental mercury by bacteria; (2) the accumulation of mercury by bacteria; and (3) the toxicity of elemental mercury to bacteria.

The stability of elemental mercury in sterile, aerobic systems is affected by the nature of the organic carbon in the medium (Table 5). Elemental mercury is stable in a sterile, basal salts medium[30] containing glucose, but is slowly oxidized in the basal salts medium supplemented with 0.1% yeast extract. Jernelöv[35] has also concluded that elemental mercury should be oxidized in natural waters by organic materials, but no reaction rates were provided.

In this investigation, B. subtilis, and B. megaterium were studied in the basal salts medium supplemented with yeast extract; E. coli, P. fluorescens, P. aeruginosa, and Citrobacter were studied in the basal salts medium supplemented with glucose.

All of the bacteria growing in the test media stimulated the oxidation of elemental mercury (Table 6), as determined

Table 4. FATE OF ELEMENTAL MERCURY IN WATER-SEDIMENT SYSTEMS.

System	Total Incubation Period (Days)	Mercury in Sediments, µg/g	
		Total Mercury	Methylmercury
Control	33	0.12	0.0
Hg^{o} (4-day exposure)	33	1.12	0.006
Hg^{o} (33-day exposure)	33	15.6	0.017

Table 5. COMPARISON OF THE STABILITY OF ELEMENTAL MERCURY IN TWO GROWTH MEDIA.

Time (h)[a]	Basal Salts Medium		Basal Salts Medium + Yeast Extract	
	Elemental Mercury (µg/l)	Total Mercury (µg/l)	Elemental Mercury (µg/l)	Total Mercury (µg/l)
0	57.0 (S=6.1, N=10)	57.5 (S=10.6, N=10)	56.5 (S=5.6, N=9)	104.9 (S=19.9, N=9)
48	56.3 (S=4.3, N=10)	52.5 (S=4.3, N=9)	35.0 (S=4.9, N=9)	102.1 (S=22.9, N=9)

[a] Hours represent time elapsed after removal of Hg^{o} globule.

Table 6. CONCENTRATIONS OF ELEMENTAL MERCURY AND MERCURIC ION AT THE 0[a] AND 48-HOUR SAMPLING TIMES

Organism	Elemental Mercury, µg/l		Mercuric Ion, µg/l	
	0-Hour	48-Hour	0-Hour	48-Hour
P. aeruginosa	58.6 (S=7.1, N=6)	54.0 (S=5.5, N=6)	0.6 (S=1.3, N=6)	3.8 (S=4.4, N=6)
P. fluorescens	54.6 (S=6.4, N=8)	38.4 (S=9.4, N=7)	0.3 (S=0.7, N=7)	6.7 (S=3.7, N=7)
Citrobacter sp.	57.6 (S=8.6, N=4)	36.8 (S=4.5, N=4)	1.6 (S=1.5, N=4)	1.5 (S=1.0, N=4)
E. coli	59.2 (S=2.8, N=4)	34.0 (S=9.0, N=4)	1.1 (S=1.5, N=4)	2.1 (S=2.8, N=4)
B. subtilis	49.8 (S=8.7, N=4)	7.2 (S=6.1, N=4)	11.9 (S=8.5, N=4)	8.2 (S=10.3, N=4)
B. megaterium	57.9 (S=7.1, N=10)	0.1 (S=0.2, N=10)	9.6 (S=6.7, N=10)	1.8 (S=2.3, N=10)

[a]Mercury analyses were completed on samples removed prior to adding the inoculum.

by paired "t" tests ($\alpha = 0.05$). The amount of mercury oxidized by the bacteria ranged from small amounts (P. aeruginosa) to nearly 100% (B. megaterium). The small amount of oxidation by P. aeruginosa, P. fluorescens, and E. coli is expected since these species have been shown to reduce mercuric ion to elemental mercury[20, 36].

The elemental mercury oxidized by the bacteria was not quantitatively recovered as mercuric ion (Table 6). Apparently most of the oxidized mercury in the growth systems is complexed in such a manner that it is not released during reduction by stannous chloride.

Consequently, only two of the populations, P. fluorescens and B. megaterium significantly changed the concentrations of mercuric ion in the medium within the 48-hour incubation period. P. fluorescens increased the concentration of mercuric ion, B. megaterium decreased it. The differences between these two cultures may be due either to the different growth kinetics exhibited by the organisms (P. fluorescens grew poorly in the presence of elemental mercury, therefore there were less cells to bind the mercuric ion), or to different growth media in which the cultures were studied.

The elemental mercury that was oxidized in these systems was not transformed to methylmercury. After an incubation of 48 hours, all cultures were analyzed for methylmercury, but none was detected (detection limit, 0.6 µg/l). This differs from results reported by Vonk and Sijpesteijn[12] who reported that some of these genera can produce small amounts of methylmercury from mercuric chloride. The different results may be explained by the fact that different media, mercury sources, incubation periods, and analytical procedures were used in the two studies.

Mercury added to these systems accumulated in the bacterial cells (Table 7). In these six cultures, the percentage of the total mercury in the system associated with the bacterial biomass ranged from 18.6 to 43.2%. Generally those organisms growing in the basal salts medium with glucose contained less mercury than those growing with the yeast extract. The concentration factors for accumulation of mercury were 222, 196, and 1202 for Citrobacter, E. coli, and P. fluorescens, respectively (Table 8).

Growth of bacterial populations may be affected by elemental mercury. Table 9, a summary of $\log_{10}$ changes in

Table 7. MERCURY ACCUMULATION BY BACTERIA DURING A 48-HOUR GROWTH PERIOD.

Organism	Total Mercury, µg/l	Cell-Associated Mercury, µg/l	% Cell-Associated
P. aeruginosa	47.4 (S=3.87, N=5)	No Growth	
P. fluorescens	56.4 (S=10.4, N=6)	10.5 (S=10.6, N=4)	18.6
Citrobacter sp.	45.9 (S=10.7, N=4)	12.3 (S=4.0, N=4)	26.8
E. coli	57.6 (S=2.8, N=2)	13.5 (S=2.1, N=2)	23.4
B. subtilis	100.8 (S=14.5, N=4)	43.1 (S=26.4, N=3)	42.7
B. megaterium	116.4 (S=22.2, N=10)	50.3 (S=19.6, N=7)	43.2

Table 8. MERCURY CONCENTRATION FACTORS OF BACTERIA GROWN IN A BASAL SALTS MEDIUM CONTAINING ELEMENTAL MERCURY

Organism	Elemental Mercury in Medium µg/l	Mercury in Cells[a] ng/gram	Concentration Factor[b]
E. coli	57.4	11,250	196
P. fluorescens	54.6	65,630	1,202
Citrobacter	57.6	12,810	222

[a]Mercury content of cells was estimated by measuring the mercury concentration in a centrifuged 48-hour culture of bacteria and using the assumption that 10^6 bacteria weigh 1 µg (wet weight).

[b]The concentration factor was obtained by dividing the mercury concentration of the cells by the mercury concentration of the medium.

Table 9. POPULATION CHANGES[a] OF BACTERIA IN MEDIA WITH AND WITHOUT ELEMENTAL MERCURY

Organism	$\log_{10}$ Population Changes	
	Control	Elemental Mercury
P. aeruginosa	+ 4	- 2
P. fluorescens	+ 4	+ 3
Citrobacter sp.	+ 3	+ 3
E. coli	+ 3	+ 3

[a]Expressed as $\log_{10}$ changes between the 0 and 48-hour sampling times.

the test populations between the 0 and 48-hour sampling periods, shows that elemental mercury killed P. aeruginosa and decreased the growth rate of P. fluorescens. This phenomenon demonstrates that the impact of elemental mercury on a complex aquatic system cannot be accurately predicted with a study of only a few microbial cultures.

PHASE III -- MERCURY DISTRIBUTION IN AQUATIC BIOTA

Dragonfly and damselfly nymphs, beetles, water bugs, snails, tadpoles, and mosquito fish, Gambusia affinis, were analyzed for total and methylmercury (Appendix C).

The data (Table 10) permitted the following conclusions about the levels of total mercury in biota from a stream receiving a continuous input of mercuric ion:

1. The levels of total mercury in dragonfly nymphs and damselfly nymphs were much higher than levels found in the other biota.

2. The levels of total mercury ranged greatly between the groups of biota.

3. When several samples of a single species were analyzed for total mercury, the results ranged widely.

If all the data for total mercury are considered, the bottom dwelling organisms (damselfly nymphs, dragonfly nymphs, and tadpoles) appear to have an average mercury level ($\bar{x}$ = 12.41 µg/g) higher than the average calculated for those forms living in the water column (corixids, dytiscids, hydrophilids, notonectids, and mosquito fish, $\bar{x}$ = 2.47 µg/g). If the biota are grouped according to feeding habits instead of habitat, then the carnivores (dragonflys, damselflys, notonectids, dytiscids, and mosquito fish) contained on the average more mercury ($\bar{x}$ = 10.15 µg/g) than the herbivores and detritivores (corixids, hydrophilids, snails, and tadpoles, $\bar{x}$ = 2.98 µg/g). Although small sample sizes do not permit extensive statistical treatment of these data, these calculations suggest that both the habitat and food habits could affect the mercury concentrations in these aquatic biota.

Quantitation of methylmercury in aquatic biota in the same stream showed that

1. the mosquito fish contained a greater portion of the total mercury as methylmercury than did most of the other biota;

Table 10. MERCURY IN AQUATIC BIOTA EXPOSED TO A CONTINUOUS INPUT OF MERCURIC ION

Biota	Number of Analyses[b]	µg Mercury/g[a] Total	Methyl	%Methyl
Libellulidae-*Neurocordulina alabamensis* (Dragonfly Nymphs)	1	14.40	0.06	0.41
	1	14.20	0.06	0.42
Coenagrionidae-*Argia sp.* (Damselfly Nymphs)	1	22.20	0.10	0.45
	1	23.20	0.06	0.25
Notonectidae-*Notonecta indica* (Back Swimmers)	1	1.30	0.09	6.92
Corixidae-*Hesperocorixa sp.* (Water Boatmen)	1	0.06	0.01	16.67
	1	0.33	--	--[c]
Dytiscidae (Predaceous Diving Beetles)	1	1.00	0.17	17.00
Hydrophilidae-*Tropisternus sp.* (Water Scaverger Beetles)	1	2.05	0.01	0.49
Lymnaeidae-*Lymnaea sp.* (Pond Snails)	3	2.14	0.01	0.47
	2	1.80	--	--
Physidae-*Physa sp.* (Pond Snails)	1	2.40	0.01	0.42
	3	8.20	0.04	0.49
Ranidae-*Rana sp.* (Tadpoles)	3	2.08	0.01	0.48
	3	4.36	0.03	0.69
	1	6.41	0.00	0.00
Poeciliidae-*Gambusia affinis* (Mosquito fish)	3	2.42	0.28	11.57
	2	3.35	--	--
	1	9.28	0.37	3.99

[a]ug Mercury per gram of wet tissue homogenate.

[b]Numbers in this column refer to single, duplicate, or triplicate total mercury analyses performed on one pooled sample.

[c]Analysis not done.

2. except for the mosquito fish, all groups of the biota had similar concentrations of methylmercury.

The low percentage of methylmercury in the mosquito fish compared to those for bass and crappie muscle tissue may be a result of (1) high inorganic mercury content of fish gut contents, (2) physiological differences among fish species, or (3) the age of the fish. It is unlikely that the low percentages were caused by inadequate extraction techniques, since analyses in our laboratory with muscle tissue from large-mouth bass and black crappie yielded high values.

The data do not indicate the source of the methylmercury acquired by the mosquito fish. If methylmercury were taken up from the water, then other gilled forms, especially the tadpoles, could be expected to have high levels. If methylmercury were taken up in the food, then the predaceous forms such as damselfly and dragonfly nymphs and mosquito fish could be expected to have similar concentrations of methylmercury. Neither pattern was evident in these samples.

No methylmercury was detected in a bottom community of algae, fungi, and bacteria exposed continuously to 1 µg/l mercuric ion, even though their total mercury content was 42 µg/g (wet weight).

Concentrations of total mercury in organisms collected from the contaminated streams were 10 to 100 times the total mercury concentrations of biota from the stream receiving no mercuric ion (Table 11); except for trace amounts in the mosquito fish, no methylmercury was detected in biota from the control stream.

Biota collected two weeks after the mercury input was stopped (Table 12) showed significantly lower total mercury concentrations than biota found in the stream while mercuric ion was being added continuously; but there was no significant difference in the methylmercury concentration in the same species collected two weeks after mercury input was stopped.

Table 11. MERCURY IN AQUATIC BIOTA FROM INPUT AND CONTROL AREA

Biota	µg Mercury/g[a]			
	Total		Methyl	
	Input Area[b]	Control Area[c]	Input Area[b]	Control Area[c]
Libellulidae (Dragonfly Nymphs)	14.30	0.21	0.06	0.00
Corixidae (Water Boatmen)	.20	0.02	0.01	0.00
Rana sp. (Tadpoles)	4.28	0.10	0.01	0.00
Gambusia affinis (Mosquito fish)	5.02	0.06	0.32	trace[d]

[a]µg Mercury per gram of wet tissue homogenate.

[b]Average of total mercury and methylmercury entries in Table 1.

[c]Results of one analysis.

[d]Below detectability of extraction procedure (0.01 µg/g).

Table 12. MERCURY IN AQUATIC BIOTA BEFORE AND AFTER DISCONTINUED INPUT

Biota	µg/Mercury/g[a]					
	Total		Methyl		% Methyl	
	Before[b]	After[c]	Before[b]	After[c]	Before[b]	After[c]
Libellulidae (Dragonfly Nymphs)	14.30	0.29	0.06	0.04	0.42	13.79
Corixidae (Water Boatmen)	0.20	0.09	0.01	0.03	5.00	33.33
Dytiscidae (Predaceous Diving Beetles)	1.00	0.42	0.17	0.10	17.00	23.81
Hydrophilidae (Water Scavenger Beetles)	2.05	0.77	0.01	0.02	.49	2.60

[a]µg Mercury per gram of wet tissue homogenate

[b]Average of total mercury, methylmercury, percent methyl entries in Table 1.

[c]Results of one analysis.

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APPENDIX A

TRANSFORMATIONS OF MERCURY IN WATER-SEDIMENT SYSTEMS

A variety of mixed-culture environments were used to study the impact of organic carbon, oxygen, and the form and concentration of mercury on microbial transformations of mercury in water-sediment systems.

To determine the effects of carbon and mercuric ion concentration on mercury transformations in anaerobic environments, different concentrations of carbon (0 and 10 g/l calcium acetate) and mercuric chloride (0, 10, and 50 mg/l) were incubated in flask systems (Figure 1) containing 500 ml of medium[25] and 100 grams of homogenized sediments. The flasks were bubbled with nitrogen gas at a rate of 20 ml/min at 25°C for the duration of the 25-day incubation, with the effluent gases trapped by methods of Kimura and Miller[29]. Elemental mercury was quantitated in the effluent gas; total and methylmercury were quantitated in the sediments.

The impact of oxygen on the fate of mercury was determined by studying mercury transformations in a sediment incubated anaerobically for 14 days, and then aerobically for 14 days. One thousand grams of a farm pond sediment, 5 liters of Barker's Medium[37], 250 mg of mercury as mercuric chloride, 50 g calcium acetate, and 5 grams of glucose were incubated at 32°C with nitrogen gas flowing at a rate of 20 ml/min for days 1-14, and with air flowing at a rate of 20 ml/min for days 15-28. During the aeration, the water-sediment was continuously mixed at 100 rpm; the system was thoroughly mixed for 10 minutes (800 rpm) before collecting water and sediment samples. The effluent gas was passed through traps to catch dimethylmercury, elemental mercury, and any other forms of mercury released; the water and sediments were periodically analyzed for methylmercury and total mercury. The effluent air was also analyzed for methane and carbon dioxide.

The fate of elemental mercury in water-sediment systems was determined in an apparatus designed by Holm and Cox[30]. To each system containing 150 grams of sediment, (sterile or non-sterile, depending on the test), were added 500 ml of a 25% soil extract[38] and 0.25 g K_2HPO_4, and the pH was adjusted to 7.5. Elemental mercury was placed in the closed system and allowed to equilibrate with the medium for 0, 4, or 33 days, the total incubation period in all cases lasting 33 days at 25°C. Total mercury, methylmercury, and elemental mercury were quantitated in the water and sediments of the test systems.

APPENDIX B

THE FATE OF ELEMENTAL MERCURY IN MICROBIAL GROWTH SYSTEMS.

Six pure cultures of bacteria (E. coli, P. fluorescens, P. aeruginosa, Citrobacter, B. subtilis, and B. megaterium) were used to study the fate of elemental mercury in microbial growth systems.

Each of the mercury flasks[30] received 500 ml of either a basal salts medium[25] or a basal salts medium containing 0.1% yeast extract, and was sterilized at 121°C for 15 minutes. After the flasks cooled, elemental mercury globules were added to the mercury holder of selected flasks; and the systems were equilibrated. After 48 hours, the mercury globule was removed from the test flasks and sterile glucose (0.25%) was added to the flasks not containing yeast extract. Inocula of the appropriate test bacterium were added (usually to a concentration of 10^6/ml), and flasks were incubated at 25°C at 125 rpm for 48 hours.

B. subtilis and B. megaterium were studied in medium supplemented with yeast extract; the other cultures were studied in medium supplemented with glucose.

Each experiment contained duplicate sterile controls containing elemental mercury; duplicate inoculated controls, with no mercury; and duplicate test systems receiving elemental mercury and bacteria.

At zero time (just prior to inoculation), duplicate samples were removed for mercury analyses (total, elemental, and mercuric ion), after which bacteria were added, the flask contents were mixed, and samples were removed for plate counts.

After 48 hours duplicate mercury analyses (elemental mercury, mercuric ion, total mercury, methylmercury, and bacterial-associated mercury) and bacterial counts (TGE pour plates) were completed.

APPENDIX C

MERCURY DISTRIBUTION IN AQUATIC BIOTA

Aquatic biota were collected from a drainage area containing the combined effluent from six artificial stream channels located near Aiken, South Carolina. The stream in this drainage area had a continuous flow of about 600 l/min. and included two distinct habitats. In the first area the water flowed rapidly over a rocky bottom (Rocky Creek Area). The second area was a ditch containing the backwater from the first area. Here, the water flowed slowly over a bottom with typical pond community emergent vegetation such as cattails (Cattail Ditch Area).

Low level mercuric ion concentrations of 0.01, 1.0 and 5 µg /l were maintained continuously in the artificial streams for eighteen months and then were discontinued. During mercuric ion addition the total mercury concentration in the water from both the Rocky Creek Area and the Cattail Ditch Area was approximately 0.8 µg/l. Organisms were collected from these areas while mercuric ion was being added and also two weeks after the mercury input was discontinued.

Control biota were collected from a ditch containing slow moving backwater from a constantly flowing artesian well from the same aquifer as that supplying the artificial stream channels.

The organisms were drained and homogenized in a chilled tissue grinder. Depending on size, the number of individuals incorporated into the homogenate ranged from three to thirty. Aliquots were removed and weighed wet for total and methylmercury analyses.